

**DATE:** December 21, 2000

**MEMORANDUM**

**SUBJECT:** *Atrazine* - 3<sup>rd</sup> Report of the Hazard Identification Assessment Review Committee.

**FROM:** Vicki Dellarco, Senior Scientist  
and  
Karl Baetcke, Senior Scientist  
Health Effects Division (7509C)

**THROUGH:** Jess Rowland, Co-Chair  
and  
Elizabeth Doyle, Co-Chair  
Hazard Identification Assessment Review Committee  
Health Effects Division (7509C)

**TO:** Cathy Eiden, Risk Assessor  
Reregistration Branch III/LH  
Health Effects Division (7509C)

**PC Code:** 080803

Atrazine (ATR) was evaluated by the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) in August of 1998. On May 4, 2000, HIARC re-reviewed the acute and chronic Reference Doses (RfDs) and the toxicological endpoint selection for occupational/residential exposure risk assessments. The May 4<sup>th</sup> reevaluation was based on the receipt of additional data generated by the Agency's National Health Effects Environmental Research Laboratories pertaining to ATR's potential effects on infants and children and a newly available draft ATR cancer assessment document. The potential for increased susceptibility of infants and children from exposure to ATR was also addressed in the May 4<sup>th</sup> HIARC review.

Since the May 4<sup>th</sup> HIARC review, a preliminary hazard and dose-response assessment on ATR, dated May 22, 2000 (see [http://www.epa.gov/scipoly/sap/2000/june27/finalparta\\_atz.pdf](http://www.epa.gov/scipoly/sap/2000/june27/finalparta_atz.pdf)), was presented to the FIFRA Scientific Advisory Panel (SAP) on June 27, 2000 for comment on the cancer, reproductive and developmental hazard of ATR. The final report of the June 27<sup>th</sup> SAP is now available

(<http://www.epa.gov/scipoly/sap/2000/june27/finalatrazine.pdf>). Furthermore, a Preliminary Human Health Risk Assessment for the Reregistration Eligibility Decision (RED), dated November 30, 2000, was completed and reviewed by the RARC on December 14, 2000.

The purpose of the December 18, 2000 HIARC review was to revisit the May 4<sup>th</sup> recommendations and conclusions in light of the Risk Assessment Review Committee (RARC) and SAP comments.

#### Committee Members in Attendance

Members present were: , Beth Doyle, Jonathon Chen, Jess Rowland, Brenda Tarplee, Bill Burnam, Pam Hurly, Ayaad Assaad, Yung Yang

Member(s) in absentia: Tina Levine, Elizabeth Mendez, David Nixon,

Also in attendance were: Karl Baetcke, Vicki Dellarco, Catherine Eiden, Gary Bangs of RRB 3

## 1. INTRODUCTION

Since the May 4<sup>th</sup> HIARC review, a preliminary hazard and dose-response assessment dated May 28, 2000 was presented to the FIFRA Scientific Advisory Panel (SAP) June 27, 2000. The final report of the June 27<sup>th</sup> SAP is now available. Furthermore, a preliminary Human Health Risk Assessment for the Reregistration Eligibility Decision (RED), dated November 30, 2000, was completed and reviewed by the RARC on December 14, 2000. The purpose of the December 18, 2000 HIARC review was to revisit the May 4<sup>th</sup> recommendations and conclusions in light of the RARC and SAP comments. The conclusions drawn at this meeting are presented in this report.

## 2. HAZARD IDENTIFICATION

### 2.1 Acute Reference Dose (RfD) - Female 13-50 Subpopulation

Study Selected: A weight of the evidence consideration used evidence provided by four studies: two developmental toxicity studies in rats; a rabbit developmental toxicity study and a study examining the effects of maternal ATR exposure during lactation on prostate effects in male offspring. The actual NOAEL and endpoint from which the reference dose is calculated is derived from one of the above-mentioned developmental studies in the rat - MRID 40566302.

Study #1 - MRID No.: 40566302

Executive Summary: Developmental toxicity study in Charles River CD rats (MRID 40566302). ATR (96.7%) was administered to 27 rats/dose by gastric intubation at 0, 10, 70, or 700 mg/kg/day from days 6 through 15 of gestation.

Mortality was very high for the 700 mg/kg/day animals in this study. All but 6 of the 27 females in this group died during the gestation period. Other statistically significant findings in this group included: salivation; oral and nasal discharge; ptosis; swollen abdomens; blood on the vulva; enlarged stomachs and adrenal; and discolored lungs. Body weight gains and food consumption were statistically significantly reduced throughout most of the gestation period. Pregnancy rates (85.2% for controls *vs* 96.3% high dose) and the numbers of live fetuses at c-section (mean of 12.7 per litter for controls *vs* 13.4 for the high dose) for the high dose group were comparable to controls. There were few findings in either the low or mid dose animals.

**The maternal LOAEL is 70 mg/kg/day, based on reduced body weight gain. The maternal NOAEL is 10 mg/kg/day.**

Fetal weights were statistically significantly reduced in the high dose group. Skeletal examinations were not conducted in the high dose group due to the extremely low fetal weights. Visceral and external examinations were conducted. No group, including the high dose group, displayed any findings significantly different from control values. Skeletal anomalies were observed in the mid dose group.

**The developmental LOAEL was found to be 70 mg/kg/day, based on delayed or no ossification at several sites. The developmental NOAEL is 10 mg/kg/day.**

The developmental toxicity study in the rat is classified **Acceptable-Guideline** and does satisfy the guideline requirement for a developmental toxicity study §83-3a in rats.

Study #2 - MRID No.: 41065201

Executive Summary: In a developmental toxicity study (MRID 41065201) ATR (97.6%) was administered by gavage to 104 mated female Sprague-Dawley rats, 26/dose, at dose levels of 0, 5 (LDT), 25 (MDT), 100 (HDT) mg/kg/day from days 6 through 15 of gestation.

Maternal toxicity findings were almost exclusively confined to the high dose group. Compared to controls high dose dams displayed: reduced food consumption (decreased 13%,  $p \leq 0.5$ ); reduced total body weight gain (reduced 18% during dosing period,  $p \leq 0.5$ ); reduced corrected (minus uterine weight) weight gain (reduced 20% for entire gestation,  $p \leq 0.5$ ); and increased alopecia 1/26 controls vs 5/26 high dose). One high dose animal died on gestation day 20 and salivation was noted as an observation in 18/26 high dose animals. The only observations seen outside the high dose group were: an abortion from one of the mid-dose animals on gestation day 19; a fluid-filled hollow right kidney in a mid-dose animal; and hollow discolored kidneys in a low dose animal.

**The maternal LOAEL is 100 mg/kg/day based on reduced body weight gain and food consumption. The maternal NOAEL is 25 mg/kg/day.**

The few malformations seen upon external examination of the fetuses were seen only in the control groups and clearly could not be compound related. Likewise, there was no increased incidence of visceral malformation in dosed groups vs control groups. There were no skeletal malformations observed but there was an increased incidence of incomplete ossification of various bones in the HDT. Hyoids (control fetal incidence of 11% vs 21.7% HDT), occipitals (7.7% vs 21.1%) and parietals (2.2% vs 8.4%) showed incomplete ossification. There was also an increased incidence ( $p \leq 0.05$ ) of incomplete ossification of the interparietals in all dose groups compared to controls.

Fetal body weight, number of resorptions and implantations, and live fetuses/litter were not significantly affected by ATR treatment. Exposure of gravid Sprague Dawley rats to ATR under the conditions described in this study seemed to have few embryo/fetotoxic effects.

**The developmental LOAEL is 100 mg/kg/day, based on increased incidence of delayed ossification of skull bones. The developmental NOAEL is 25 mg/kg/day.**

The developmental toxicity study (MRID 41065201) in the rat is classified **Acceptable -Guideline** and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3a) in the rat.

Study #3 - MRID Nos.: 00143006, 40566301

Executive Summary: In a developmental toxicity study (Acc. No. 254979; MRIDs 00143006, 40566301) ATR (96.3%) was administered by gavage to 76 mated female New Zealand White rabbits, 19/dose, at dose levels of 0, 1, 5, or 75 mg/kg/day, from days 7 through 19 of gestation.

Clinical signs seen in 75 mg/kg/day (HDT) animals that were considered to be related to compound treatment were stool changes (none, little or soft stool; 9/19 controls *vs* 19/19 HDT), and the appearance of blood in the cage or on the vulva (0/19 controls *vs* 4/19 HDT). Body weight gain was reduced in high dose dams and, at many time points, body weight was below day zero values. At gestation days 14, 19, 21 and 25, mean maternal body weights were 12%, 19%, 18%, and 10% below control values ( $p \leq 0.01$  for all four of these time points).

High dose animals displayed significantly reduced food consumption during treatment. During gestation days 12 to 17 the HDT average feed consumption was only 1-6 g of feed per animal per day compared to 175-182 g for the controls. The mid and low dose groups had no alterations that could be attributed to ATR exposure.

**The maternal toxicity LOAEL is 75 mg/kg/day based on decreased body weight, food consumption and increased incidence of clinical signs. The maternal toxicity NOAEL is 5 mg/kg/day.**

Increased resorptions - mean of 1.3/dam in controls *vs* 4.8/dam in HDT - ( $p \leq 0.01$ ), reduced live fetuses per litter - mean of 8.8/dam in controls *vs* 5.9/dam in HDT - ( $p \leq 0.05$ ), and increased delayed ossification of appendicular elements were observed in the high dose group. The low and intermediate groups had no fetal findings that could be attributed to compound exposure. The findings in the high dose group were determined to be secondary to maternal toxicity and thus the LOAEL and NOAEL for embryo/fetotoxicity match the maternal LOAEL and NOAEL.

**The developmental toxicity LOAEL is 75 mg/kg/day based on reduced litter size, increased resorptions and delayed ossification. The developmental toxicity NOAEL is 5 mg/kg/day**

The study is considered **Acceptable - Guideline** and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3b) in rabbit.

Study #4 - MRID No.: 45166902

Executive Summary: Hyperprolactinemia prior to puberty in male rats has been shown to lead to lateral prostate inflammation in young adult rats. One possible cause of hyperprolactinemia in immature male rats is a deficiency in milk-derived prolactin (PRL). Milk-derived PRL plays a critical role in the development of the tuberoinfundibular dopaminergic neurons (TIDA) of the hypothalamus of a developing rat. The TIDA neurons function to inhibit PRL secretion from the anterior pituitary. Organization and development of these neurons occurs mainly during the first postnatal week in the rat (Ojeda and McCann, 1974). Thus, if developing rats do not receive a sufficient amount of PRL from their mothers milk during the first week after birth, the TIDA neurons will not develop properly and may not be able to sufficiently provide an inhibitory check to PRL secretion in the adult animal. The resultant hyperprolactinemia is associated with development of prostatitis in the adult.

ATR has been shown to depress the secretion of PRL. The role of milk-derived PRL in development of the TIDA neurons in the neonatal rat hypothalamus, and the resulting hyperprolactinemia followed by lateral prostatitis that is the consequence of incomplete development of these neurons is described above. To summarize these points: without early lactational exposure to PRL, TIDA neuronal growth is impaired and elevated PRL levels are present in the prepubertal male. Hyperprolactinemia in the adult male rat has been implicated in the development of prostatitis. Thus, early lactational exposure of dams to agents that suppress suckling-induced PRL release (possibly ATR) could lead to a disruption in TIDA development in the suckling male offspring, followed by altered PRL regulation and subsequent hyperprolactinemia and prostatitis in these male offspring.

To test the hypothesis that ATR exposure of dams during lactation could initiate the above-described sequence of events, suckling-induced PRL release was measured in Wistar dams treated with ATR (by gavage, twice daily on PND 1-4 at 0, 6.25, 12.5, 25, and 50 mg/kg) or the dopamine receptor agonist bromocriptine (BROM, sc, twice daily at 0.052, 0.104, 0.208 and 0.417 mg/kg). BROM is known to suppress PRL release. Serum PRL was measured on PND 3 using a serial sampling technique and indwelling cardiac catheters.

A significant rise in serum PRL release was noted in all control females within 10 minutes of the initiation of suckling. Fifty mg/kg ATR inhibited suckling-induced PRL release in all females, whereas 25 and 12.5 mg/kg ATR inhibited this measure in some dams and had no discernible effect in others. The 6.25 mg/kg dose of ATR was without effect. BROM also inhibited suckling-induced PRL release at the two highest doses.

To examine the effect of postnatal ATR and BROM on the incidence and severity of inflammation (INF) of the lateral prostate of the offspring, adult males were examined at 90 and 120 days. No effect was noted at 90 days of age. At 120 days, both the incidence and severity of prostate inflammation was increased in those offspring of ATR-treated dams (25 and 50 mg/kg). The 12.5 mg/kg ATR and the two highest doses of BROM increased the incidence, but not severity, of prostatitis. Combined treatment of ovine PRL (oPRL) and 25 or 50 mg/kg ATR on PN1-4 reduced the incidence of inflammation observed at 120 days, indicating that this increase in INF seen after ATR alone resulted

from the suppression of PRL in the dam. Testing to determine whether there is a critical period for these effects revealed that the critical period for this effect is PND1-9.

These data demonstrate that ATR suppresses suckling-induced PRL release and that this suppression results in an increase in lateral prostate inflammation in the offspring and that the critical period for this effect is PND1-9.

**Dose and Endpoint for Risk Assessment: Developmental NOAEL = 10 mg/kg/day based on delayed or lack of ossification of several sites at 70 mg/kg/day (LOAEL), and supported by the decreased suckling induced PRL release and increased incidence of prostatitis (NOAEL 12.5 mg/kg/day, LOAEL 25 mg/kg/day).**

**Uncertainty Factor (UF): 100**

In summary, reproductive and developmental effects in various strains of rats that are associated with ATR treatment include pre-implantation and post-implantation losses, prostatitis in adult male offspring of treated lactating females, delays in vaginal opening and preputial separation, and disruption of the estrous cycle in young females. A reduction in PRL release in nursing dams is strongly associated with the development of prostatitis in male adult offspring. Decreases in serum lutenizing hormone (LH) or PRL were not observed to occur at dose-levels that led to delays in vaginal opening (50 mg/kg/day) and preputial separation (13 mg/kg/day) in the same study but it is presumed that the variability in levels of these hormones in juvenile animals preclude obtaining definitive data. On the other hand, a separate study using dams showed that a daily dose of ~13 mg/kg/day was sufficient to depress serum levels of PRL in the lactating dam. To the extent that decreased PRL levels can serve as a marker for effects on neuroendocrine control, there is a linkage between pubertal development and an effect on the hypothalamic-pituitary axis.

Comments about Study/Endpoint/Uncertainty Factor: Any of the four studies described above may be appropriate for selection of an endpoint for acute risk assessment. The developmental effects seen in the two rat and one rabbit developmental study are assumed to have the potential to occur after a single dosing. The effects seen in the open literature prostatitis paper occurs after only four days of dosing.

The lowest NOAEL seen in the above studies was 5 mg/kg/day, which is the developmental NOAEL from the rabbit developmental toxicity study (MRID 41065201). Though the NOAEL from this study would be acceptable for use as an acute RfD, HIARC notes that there was a large dose spread in this study. The mid dose tested (and the NOAEL) in this study was 5 mg/kg/day while the next highest dose tested (the highest dose tested and the LOAEL) was 75 mg/kg/day. This dose is a full 15 times higher than the mid dose tested. The large spread between 5 and 75 mg/kg/day raises the possibility that had intermediate doses between 5 and 75 been used then the NOAEL would have been higher.

Examination of the rat developmental toxicity studies indicates that intermediate doses in the rabbit study between 5 and 75 may not have shown any adverse effects. The NOAEL in both the rat studies

are greater than 5 mg/kg/day (10 mg/kg/day for MRID 40566302 and 25 mg/kg/day for MRID 41065201). The effects seen in the rabbit and two rat developmental toxicity studies are similar with all three studies demonstrating delayed or no ossification in certain cranial bones at their respective LOAELs of 75 (rabbit), 70 (MRID 40566302) and 100 mg/kg/day (MRID 41065201). Other effects on which the developmental NOAEL were based in the rabbit study - reduced litter size and increased resorptions - were not seen in either of the rat studies and are not considered to be frank malformations, or even variations. In this respect it should be noted that maternal effects were more severe at the LOAEL in the rabbit study than at the LOAELs in either of the two rat studies. The maternal LOAELs in the two rat studies were based on decreased food consumption and body. The maternal LOAEL in the rabbit study was based on clinical signs (none, little or soft stool, blood on the vulva), in addition to decreased food consumption and body weight.

HIARC also notes that an acute RfD based on a NOAEL of 10 mg/kg/day is supported by the prostatitis effects which have a NOAEL of 12.5 mg/kg/day.

$\text{Acute RfD} = \frac{10 \text{ mg/kg}}{100} = 0.1 \text{ mg/kg}$
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### **Dose and Endpoint for General Population including infants and children**

An appropriate endpoint for the general population attributable to a single exposure was not available from the oral toxicity studies including the developmental toxicity studies in rats and rabbits.

## **2.2 Chronic Reference Dose (RfD)**

As discussed in detail in the OPP May 22, 2000 hazard and dose-response document, ATR alters hypothalamic gonadotrophin releasing hormone (GnRH) release in rats. There are also some data that indicate that ATR diminishes norepinephrine in the rat hypothalamus as an initial or early site of action which in turn leads to diminished GnRH release. ATR also increases dopamine levels which can result in a diminished pituitary PRL secretion. Therefore, ATR appears to operate at the level of the hypothalamus. In both humans and rats, hypothalamic GnRH controls pituitary hormone secretion (*e.g.*, LH, PRL). The hypothalamic-pituitary axis is involved in the development of the reproductive system, and its maintenance and functioning in adulthood. Additionally, reproductive hormones modulate the function of numerous other metabolic processes (*i.e.*, bone formation, and immune, central nervous system (CNS) and cardiovascular functions). Therefore, altered hypothalamic-pituitary function can potentially broadly affect an individual's functional status and lead to a variety of health consequences. The June SAP indicated that "...it is not unreasonable to expect that ATR might cause adverse effects on hypothalamic-pituitary function in humans." Therefore, ATR's effect on ovarian cycling and the pre-ovulatory LH surge (as well as its effects on pregnancy, puberty, suckling induced

PRL release which leads to prostatitis) are viewed as neuroendocrinopathies or biomarkers indicative of ATR's ability to alter hypothalamic-pituitary function in general. It should be noted that ATR's neuroendocrine effects have been demonstrated in several strains of rats (SD, Long Evans, Wistar).

Study Selected: Six-month LH surge study

§ Special study

MRID No.: 44152102

Executive Summary: In a study to evaluate the effect of long-term ATR exposure on the proestrus afternoon LH surge (MRID 44152102) ATR, 97.1% a.i., was administered to 360 female Sprague Dawley rats in the diet. Dose levels were 0 (negative control), 25, 50, and 400 ppm (0, 1.80, 3.65, 29.44 mg/kg/day) for 26 weeks (approximately six months).

Body weight, body weight gain and food consumption were significantly ( $p \leq 0.05$ ) decreased in HDT animals compared to controls (body weight decreased 8.5% at the end of the study and food consumption decreased 3.75% for the entire study). The percentage of days in estrus were significantly increased ( $p \leq 0.01$ ) during the 21-22 and 25-26 week time periods at the HDT. Percent days in estrus were also increased during the 21-22 and 25-26 week time periods at the MDT, but the increase was only significant ( $p \leq 0.05$ ) for the 21-22 week time period. The proestrus afternoon LH surge was severely attenuated at the HDT (LH levels were actually decreased compared to baseline at most sampling time points) and less so at the MDT (maximum increase over baseline was 157% compared to maximum increase over baseline in controls of 273%). Pituitary weight were increase at the HDT (absolute weight increased 22% and weight relative to body weight was increased 28%). Pituitary weights at the other two doses were not affected. There was a slight increase at the HDT of animals displaying enlarged pituitaries (0% in controls compared to 3.4% at 29.44 mg/kg/day) and thickened mammary glands (0% in controls compared to 6.7% at 29.44 mg/kg/day). There were no other gross necropsy findings in the HDT that could be attributed to compound exposure and there were no compound-related gross pathology findings at the MDT or LDT. Selected tissues were saved for histopathology but those results have yet to be reported.

There were no compound related effects in mortality or clinical signs. The proestrus afternoon PRL surge was not affected by compound exposure at any dose. The LDT had no effects on the estrous cycle, LH or PRL surges.

**The LOAEL is 3.65 mg/kg/day, based on estrous cycle alterations and LH surge attenuation as biomarkers of ATR's ability to alter hypothalamic-pituitary function. The NOAEL is 1.8 mg/kg/day.**

This special study in the rat is **Acceptable - nonguideline**. This study does not satisfy any guideline requirements a guideline requirement.

Dose and Endpoint for Establishing RfD: **1.8 mg/kg/day based on estrous cycle alterations and LH surge attenuation at 3.65 mg/kg/day (LOAEL) as biomarkers of ATR's potential to disturb hypothalamic-pituitary function which may lead to various health consequences including reproductive disruption.**

Uncertainty Factor(s): 100

Comments about Study/Endpoint/Uncertainty Factor: The attenuation of the LH surge is considered to be an indicator of ATR's neuroendocrine mode of action or its potential to alter hypothalamic-pituitary function. This six-month study is considered adequate for use in selecting a chronic endpoint without an additional safety factor being added to account for study duration of less than 12 months.

These biomarkers of ATR neuroendocrine mode of action (i.e., LH surge attenuation and estrous cycle disruption) are considered to be applicable to the general population including infants and children given that they result from ATR's CNS mode of action. HIARC notes that this dose is the lowest NOAEL available in the toxicology database and therefore would be protective of other adverse effects, including those occurring in males, infants, and children. Therefore, a separate endpoint is not needed for this population (i.e., males, infants, and children).

This dose and endpoint replaces the previous dose and endpoint of 3.5 mg/kg/day based on decreased body weight gain and food consumption in a two-year rat bioassay selected by HIARC in 1998. The dose of 1.8 mg/kg/day for use in risk assessment would be protective of effects that occur at the higher dose of 3.5 mg/kg/day as well as protective of effects such as LH surge attenuation and estrous cycle alterations, and any effects that may be associated with alteration of these parameters.

$\text{Chronic RfD} = \frac{1.8 \text{ mg/kg/day}}{100} = 0.018 \text{ mg/kg/day}$
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## **2.3 Occupational/Residential Exposure**

### **2.3.1 Short-Term (1-30 days) Incidental Oral Exposure**

Study Selected: Developmental toxicity in rats § 83-3a

MRID No.: 40566302

Executive Summary: See Study #1 above under "Acute RfD"

Dose and Endpoint for Risk Assessment: **Maternal NOAEL = 10 mg/kg/day based on statistically significant decrease in body weight gains at 70 mg/kg/day (LOAEL).**

Comments about Study/Endpoint: Decreases in body weight gain was seen during the first 5 days of dosing. The endpoint is indicative of general or systemic toxicity and is thus appropriate for the exposure pathway of concern for infants and children. **NOTE:** The period for short-term exposure for this assessment should be 1 to 30 days. This period was selected because the intermediate-term endpoint (see section 2.3.2) requires approximately one month of exposure before onset of effects.

### **2.3.2 Intermediate-Term (30 Days to Several Months) Incidental Oral Exposure**

Study Selected: Six-month LH surge study

§ Special study

MRID No.: 44152102

Executive Summary: Same as for chronic RfD.

Dose and Endpoint for Risk Assessment: **NOAEL = 1.8 mg/kg/day based on estrous cycle alterations and LH surge attenuation at 3.65 mg/kg/day (LOAEL). These effects are biomarkers of ATR's potential to disturb hypothalamic-pituitary function which may lead to various health consequences including reproductive disruption.**

Uncertainty Factor(s): 100

Comments about Study/Endpoint: Although the 1.8 mg/kg/day is derived from a 6 month study, it is reasonable to consider for an intermediate exposure duration. This is because data from a one month study by Morseth (1996) showed effects on LH surge at 2.5 mg/kg/day (the nonrepeat bleed measures) after one month of dosing. Furthermore, in a study by Stoker et al. (2000) a NOAEL of 6 mg/kg/day and a LOAEL of 13 mg/kg/day was identified for delayed preputial separation when rats were exposed on PND 23-53. The 1.8 mg/kg/day value comes from an adult study, but is a reasonable surrogate of ATR CNS - hypothalamic disruption in children. Thus, these endpoints are considered indicative of ATR's ability to disrupt hypothalamic-pituitary function, and its potential to lead to general or systemic toxicity. The 1.8 mg/kg/day is therefore considered appropriate and protective for the population (including infants and children) of concern.

### **2.3.3 Dermal Absorption**

Dermal Absorption Factor: The committee recommended a dermal absorption factor of 6% (rounded up from 5.6%). This factor is based on a human study (MRID 44152114) in which 10 human volunteers were exposed to a single topical dose of [triazine ring-U-<sup>14</sup>C]ATR (94.3-96.3% a.i., 98.0-98.4% radiochemical purity) at 6.7 (4 volunteers) or 79 : g/cm<sup>2</sup> (6 volunteers) for 24 hours; equivalent to 0.1667 and 1.9751 mg of [14C] ATR for the low and

high doses, respectively. After 24 hours the ATR was removed and determination of percent absorbed occurred was determined 168 hours (7 days) after the commencement of exposure. The maximum percent absorbed in this study was 5.6% of the dose in the lower dose group. Because the maximum percent absorbed is being used and because an ample amount of time (168 hours) was allowed for absorption to occur, 6% is deemed to be a protective estimate of dermal exposure.

A rat (MRID 43314302) dermal penetration study was also available in which 21.6% absorption was observed. A comparison of the two studies reveals a similar dose used in each study. In the rat study, 0.1 mg/kg was applied to the skin for 10 hours. Absorption was measured at 82 hours following the application (10/82). The human study had a similar dose of 0.067 mg/kg left on the skin for 10 hours with measurement 168 hours following the start of exposure (10/168).

Because both rat and human dermal absorption studies are available, a "Rat:Human Dermal Penetration Factor" can be calculated. As noted above, humans have lower dermal absorption of ATR than rats. To account for this species difference, dermal NOAELs developed in rats may be multiplied by a rat:human dermal penetration factor. The rat:human dermal penetration factor is calculated by dividing the dermal absorption in the rat by the dermal absorption in the human. In this case the dermal absorption in the rat is taken to be 22% (the 21.6% from the 0.01 10/82 hours dose in the above described rat dermal penetration study [MRID 43314302] rounded up to 22%). This value is selected because it represents the highest dermal absorption value seen following a 10 hour exposure (approximating a typical workday) in the rat study. The value selected for the denominator for the ratio (the human portion) was 6%. This was the highest percent absorbed in this study (MRID 44152144).

$$\text{Rat:Human Dermal Penetration Factor} = \frac{22}{6} = 3.6$$

This factor is multiplied by the NOAEL for exposure scenarios in which endpoints from a dermal study are used. Exposure scenarios whose endpoints are derived from oral studies will use a 6% dermal absorption factor.

#### **2.3.4 Short-Term Dermal (1-30 days) Exposure**

Study Selected: 21-day dermal toxicity in the rabbit

§82-2

MRID No.: 42089902

Executive Summary: ATR technical (97.6%) was administered dermally to 30 New Zealand White rabbits for 6 hours/day for 25 days. Dose levels were 0, 10, 100 or 1000 mg/kg/day (5 rabbits/sex/dose).

The increased absolute and relative spleen weights in high-dose animals were not accompanied by histological findings; however, statistically significant reductions in red blood cell counts and hematocrit levels were noted in high dose females. Further findings included statistically significant ( $p < 0.01$ ) reductions in total protein and chloride in males and significantly increased cholesterol and triglyceride levels in females.

Dermal application of the test material resulted in minimal to moderate acanthosis, hyperkeratosis, and focal subacute inflammation of treated skin in high-dose females. Dermal irritation included limited to slight (grade 1) erythema and scaling in one high dose female at days 17 - 25. Erythema (grade 1) was observed in one high dose male at day 18.

The study authors reported a NOAEL of 10 mg/kg/day and a LOAEL of 100 mg/kg/day, based on slight transient reductions in mean percent body weight gain in mid dose females at days 7 and 14. Because the reductions in female body weight gain at 100 mg/kg/day were slight, not statistically significant, transient, and without significant reductions in food consumption and absolute body weight, the reviewers assessed that the changes were of equivocal biological importance.

**The NOAEL for systemic toxicity is 100 mg/kg/day. The LOAEL is 1000 mg/kg/day based on statistically significant reductions in food consumption, mean body weight, and percent weight gain in both sexes, statistically significantly increased absolute and relative spleen weights in both sexes, and slight changes in excretion (i.e. few and/or mucoid feces).**

This study is classified **Acceptable - Guideline**. This study satisfies the Guideline series 82-2 requirements for a 21-day dermal toxicity study in the rabbit.

Rat:Human Dermal Penetration Factor: 3.6

Dose and Endpoint for Risk Assessment: **360 mg/kg/day.**

This value is derived by multiplying the study NOAEL of 100 mg/kg/day (based on reduced food consumption, mean body weight, body weight gain, increased spleen weights at the LOAEL of 1000 mg/kg/day) by the dermal penetration factor of 3.6.

Comments about Study/Endpoint: This study is appropriate because the duration and route of exposure (21-day, dermal) match the duration and route of exposure (up to one month, dermal) in the short-term dermal risk assessment. **NOTE:** The period for short-term exposure for this assessment should be 1 to 30 days. This period was selected because the intermediate-term

endpoint (see section 2.3.5) requires approximately one month of exposure before onset of effects.

### **2.3.5 Intermediate-Term Dermal (30 Days to Several Months) Exposure**

Study Selected: Six-month LH surge study

§ Special study

MRID No.: 44152102

Executive Summary: See above under "Chronic RfD"

Dose/Endpoint for Risk Assessment: 1.8 mg/kg/day based on estrous cycle alterations and LH surge attenuation at 3.65 mg/kg/day (LOAEL) as biomarkers of ATR's potential to disturb hypothalamic-pituitary function which may lead to various health consequences including reproductive disruption.

Comments about Study/Endpoint: The endpoint of concern was seen after 6 months of exposure and is appropriate for this exposure period of concern. The 21-day dermal study was not selected since estrous cycle evaluations and LH measurements (both of which have been shown to be very sensitive endpoints following ATR exposure) were not performed in this study. Since an oral NOAEL was selected, the 6% dermal absorption factor should be used in route-to-route extrapolation.

### **2.3.6 Long-Term Dermal (Several Months to Life-Time) Exposure**

Study Selected: Six-month LH surge study

§ Special study

MRID No.: 44152102

Executive Summary: See above under "Chronic RfD"

Dose/Endpoint for Risk Assessment: 1.8 mg/kg/day based on estrous cycle alterations and LH surge attenuation at 3.65 mg/kg/day (LOAEL).

Comments about Study/Endpoint: This study was also used to derive the Chronic RfD. See the comments under section 2.2 above. Since an oral NOAEL was selected, the 6% dermal absorption factor should be used in route-to-route extrapolation.

### **2.3.7 Inhalation Exposure (All Durations)**

With the exception of an acute inhalation study, no inhalation studies are available for evaluation. Therefore the HIARC selected the oral NOAELs for inhalation risk assessments.

Since an oral dose is used, risk assessment should follow the route-to-route extrapolation as below:

- Step I. The inhalation exposure component (i.e., : g a.i./day) using 100% absorption rate (default value) and application rate should be converted to an equivalent oral dose (mg/kg/day).
- Step II. The dermal exposure component (mg/kg/day) using a 6% dermal absorption rate and application rate should be converted to an equivalent oral dose. This dose should then be combined with the oral equivalent dose in Step I.
- Step III. The combined oral equivalent dose from Step II should then be compared to the oral NOAELs to calculate MOEs. The NOAELs are as follows:

For short-term exposure:	10.0 mg/kg/day
For intermediate-term exposure:	1.8 mg/kg/day
For long-term exposure:	1.8 mg/kg/day

### **2.3.8 Margins of Exposure for Occupational/Residential Risk Assessments**

The level of concern for dermal and inhalation occupational exposure is an MOE of 100.

The MOEs for residential exposure risk will be determined by the FQPA SF committee.

### **2.3.9 Recommendation for Aggregate Exposure Risk Assessments**

For acute aggregate exposure, the high end value from food plus water should be combined and compared to the RfD.

For short-term aggregate risk assessment, the oral, dermal and inhalation risk estimates can be combined given the common endpoint of decreased body weight gain seen in the oral (maternal), dermal (systemic and inhalation (oral equivalent) routes of exposure.

For intermediate- and long-term aggregate risk assessment, exposure from these routes can be aggregated since oral equivalents were used for dermal and inhalation exposures given the common endpoint of concern: attenuation of the LH surge used as a biomarker indicative of disruption of hypothalamic-pituitary function.

## **3 CLASSIFICATION OF CARCINOGENIC POTENTIAL**

### **3.1 Combined Chronic Toxicity/Carcinogenicity Study in Rats**

MRID Nos.: 00158930; 42085001; 42204401; 44544701

Discussion of Tumor Data: Several chronic bioassays in the Sprague Dawley rat (MRIDs shown above) have demonstrated that chronic ATR exposure is associated with an increased incidence and/or an earlier onset of mammary tumors. There is also limited evidence (primarily from a single chronic bioassay) that ATR exposure may be associated with an earlier onset of pituitary adenomas.

Adequacy of the Dose Levels Tested: The dose levels tested were adequate to determine the carcinogenic potential of ATR.

### **3.2 Carcinogenicity Study in Mice**

MRID No. : 40431302

Executive Summary: In an oncogenicity study (MRID 40431302), ATR, (purity not given) was administered to CD-1 mice, 59-60/sex/dose, in the diet at dose levels of 0, 10, 300, 1500 and 3000 ppm (male/female mean daily dose 0/0, 1.4/1.6, 38.4/47.9, 194.0/246.9, 385.7/482.7 mg/kg/day) for 91 weeks. Female mice in the 300, 1500 and 3000 ppm groups received a daily ATR dose about 25% higher than their counterpart males. No dose-related increases in neoplasms were observed. The dose response curve seemed adequate since toxic effects, such as a decrease in mean body weight of both sexes and an increase in cardiac thrombi in the females, are seen at both 1500 and 3000 ppm, while no dose-related toxic effects are seen at 10 and 300 ppm. In addition to the toxic effects just mentioned, the 3000 ppm animals of both sexes also displayed decreases in food consumption and decreases in RBC, hematocrit, and hemoglobin concentration. Female mice, but not males, at 3000 ppm showed decreased mean group brain and kidney weights and decreased percentages of neutrophils and lymphocytes. There was also an increase in mortality ( $p < 0.05$ ) in 3000 ppm females, but not males, with only 25% of the females surviving *vs* 39-43% of the females surviving in the other female dose groups.

The cardiac thrombi found at both 1500 and 3000 ppm may have contributed to unscheduled female deaths during the course of the study. The incidence of unscheduled death in mice with cardiac thrombi is statistically significantly different from the incidence of unscheduled death in mice from control groups. The occurrence of cardiac thrombi must be considered a severe effect.

**The LOEL is 1500 ppm (222.0 mg/kg/day), based on decreased body weight gain in both sexes and increased cardiac thrombi in the females. The NOEL is 300 ppm (43 mg/kg/day).**

At the doses tested, there was not a treatment-related increase in tumor incidence when compared to controls. Dosing was considered adequate due to the occurrence of decreased body weight gain and cardiac thrombi.

This carcinogenicity study in the mouse is **Acceptable-Guideline**, and does satisfy the guideline requirement for a carcinogenicity study in the mouse.

Discussion of Tumor Data: There was no evidence of carcinogenicity in CD-1 mice following exposure to ATR for 91 weeks.

Adequacy of the Dose Levels Tested: Dose levels tested were adequate to determine carcinogenic potential

### **3.3     Classification of Carcinogenic Potential**

In 1987, the HED Cancer Peer Review Committee (CPRC) classified ATR as a Group “C” carcinogen (possible human carcinogen) and recommended a linear low dose approach ( $Q_1^*$ ) for human risk characterization. The CPRC met again on June 6, 1988 and September 29, 1988 and reaffirmed the Group C classification.

In 1997, the HED Cancer Assessment Review Committee (CARC) evaluated the carcinogenic potential of ATR and discussed mode of action data submitted by the Registrant in regards to the ability of ATR to produce mammary tumors in Sprague Dawley rats. A document (OPP document May 22, 2000) was prepared and, presented to the Science Advisory Panel (SAP) in June 27, 28 and 29th, 2000. A final Cancer Peer Review memorandum was prepared, dated December 2000, which considered the final report of the June SAP. **The CARC concluded that ATR should be classified as “not likely” to be carcinogenic to humans, given that ATR’s neuroendocrine mode of action essentially accelerated the reproductive aging process in SD female rats (and other strains of rats with a similar process) of reproductive senescence leading to a hormonal environment conducive to mammary gland tumor development. Although ATR exposure is not associated with apparent cancer consequences in humans, a potential for noncancer effects due to its ability to disrupt hypothalamic-pituitary function can not be discounted. This view is consistent with the June SAP report.**

## **4     MUTAGENICITY**

ATR has not been found to be mutagenic in bacteria and does not cause unscheduled DNA synthesis in primary rat hepatocytes. ATR did not induce clastogenicity in the mouse micronucleus assay. ATR was negative in a mouse Dominant-Lethal Assay.

An extensive review of more than 50 mutagenicity studies using ATR, and ATR metabolites is included as a chapter in the most recent cancer peer review document. This document can be accessed at the internet address shown in the previous paragraph.

## (I) Gene Mutation

In a reverse gene mutation assay in bacteria (MRID 40246601), strains TA 98, 100, 1535 and 1537 of S. typhimurium were exposed to ATR (98.2% a.i.), in dimethylsulfoxide, at concentrations of 0, 20, 78, 313, 1250, and 5000 µg/plate. Tests were conducted in the presence and absence of mammalian metabolic activation S9 fraction of Tif:RAIf rats treated with Aroclor 1254. ATR was tested up to the limit concentration, 5000 µg/plate. The positive controls did induce the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

This study is classified as **Acceptable - Guideline**. It does satisfy the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

### (ii) Structural Chromosomal Aberrations

A mouse bone marrow micronucleus test was conducted using Tif:MAGF mice (MRID 40722301). The test consisted of two parts. The first portion consisted of 24 male and 24 female mice being dosed with 2250 mg/kg ATR (98.2% a.i.). Eight animals of each sex were then sacrificed at 16, 24 or 48 hours following treatment. The second portion of the study 24 mice, 8/sex/dose, were treated with ATR (98.2% a.i.) at doses of 562.5, 1175, 2250 mg/kg. Bone marrow cells were harvested at 24 hours post-treatment. The vehicle in both portions of the study was carboxymethyl cellulose. Exposure in both portions of the study was accomplished by a single gastric intubation. There were no signs of cytotoxicity in bone marrow erythropoiesis seen either portion of the study. However, the high dose was clearly toxic since 7 of the 32 females which received the high dose died prematurely. ATR was tested at an adequate doses being that these were doses that induced death in mice. The positive control induced the appropriate response. **There was not a significant increase in the frequency of micro nucleated polychromatic erythrocytes in bone marrow after any treatment time or dose.**

This study is classified as **Acceptable - Guideline**. It does satisfy the requirement for FIFRA Test Guideline 84-2 for *in vivo* cytogenetic mutagenicity data.

### (iii) Other Genetic Effects

In an unscheduled DNA synthesis assay (MRID 42547105), primary rat hepatocyte cultures were exposed to ATR, (97.1% a.i.), in dimethyl sulfoxide at concentrations of 15, 46, 139, 417, 835, and 1670 µg/ml for 16-18 hours. ATR was tested up to precipitating concentrations, 139 µg/ml. The positive controls did induce the appropriate response. **There was no evidence that unscheduled DNA synthesis, as determined by nuclear silver grain counts, was induced.**

This study is classified as **Acceptable - Guideline**. It does satisfy the requirement for FIFRA Test Guideline 84-2 for other genotoxic mutagenicity data.

In a mouse dominant lethal assay (MRID 42637003), groups of 30 male Tif: MAGf (SPF) mice were treated orally by gavage with ATR technical (97.1% a.i., batch #SG8029BA10) at doses of 0, 500, 1000, 2000, or 2400 mg/kg body weight in a volume of 10 ml/kg. The vehicle was corn oil. Starting immediately after dosing, each male was mated with 2 untreated females per interval for days 1-4, days 4-8, and days 8-12. Each male was then mated with 2 untreated females per week for weeks three through eight.

ATR technical was tested at an adequate dose. There were signs of toxicity after dosing as evidenced by piloerection and decreased locomotor activity. The females were sacrificed on gestation day 13-15 and the uteri examined for the number of alive, early, and late dead embryos and resorptions. Cyclophosphamide served as the positive control. There was no significant difference between the control group and treated groups with respect to post-implantation mortality of embryos. Under the conditions of this study ATR technical did not induce dominant lethal mutations in male mice at doses as high as 2400 mg/kg.

This study is classified as **Acceptable - Guideline**. It does satisfy the requirement for FIFRA Test guideline 84-2 for rodent dominant lethal data

## **5 FQPA CONSIDERATIONS**

### **5.1 Adequacy of the Data Base**

The toxicology database for ATR was considered adequate by the HIARC for consideration of factors under FQPA.

### **5.2 Neurotoxicity**

Acute and subchronic neurotoxicity studies are not available for ATR. Special studies submitted by the registrant (MRIDs 44152102 and 43934406) and published in the open literature (Cooper, et al. 2000. Atrazine disrupts the hypothalamic control of pituitary-gonadal function. *Tox. Sci.* 53: 297-307 [MRID 45166902]) provide evidence of ATR-associated neurotoxicity. The neurotoxicity seen in these studies was a central nervous system (CNS) toxicity (specifically, neurotransmitter and neuropeptide alterations at the level of the hypothalamus).

### **5.3 Developmental Toxicity**

One rabbit and two rat developmental studies are available for evaluation. The executive summaries for these three studies are shown above under "Acute RfD: Study #'s 1, 2, and 3."

### **5.4 Reproductive Toxicity**

A two-generation study is available for evaluation.

In a 2-generation reproduction study (MRID 40431303) ATR, (purity not specified but said to be technical grade) was administered to 240 Charles River (CRCD, VAF/PLUS) rats 30/sex/dose in the diet at dose levels of 0, 10, 50, and 500 ppm. There was very little variation in test article consumption between generations; the F<sub>0</sub> and F<sub>1</sub> males had similar test article consumption during the 70-day pre-mating period as did the F<sub>0</sub> and F<sub>1</sub> females. The average values for the two generations are 0, 0.75, 3.78, 39.0 mg/kg/day for males and 0, 0.86, 3.70, 42.8 mg/kg/day for females. Test article consumption for the F<sub>0</sub> and F<sub>1</sub> generation females during their gestation period did not vary greatly between generation. Mean compound consumption for both generations were 0, 0.66, 3.33 and 35.43 mg/kg/day.

Parental body weights, body weight gain, and food consumption were statistically significantly reduced at the 500 ppm dose (HDT) in both sexes and both generations throughout the study. Compared to controls, body weights for F<sub>0</sub> HDT males and females at 70 days into the study were decreased by 12% and 15%, respectively while F<sub>1</sub> body weight for the same time period was decreased by 15% and 13% for males and females, respectively. The only other parental effect which may have been treatment related was a slight, but statistically significant, increase in relative testes weight which occurred in both generations of the HDT. **The parental LOAEL is 500 ppm (39 mg/kg/day in males, 42.8 mg/kg/day in females) based on decreased body weights, body weight gains and food consumption. The NOAEL is 50 ppm (3.78 mg/kg/day in males, 3.7 mg/kg/day in females).**

There did not appear to be any offspring toxicity in females from compound exposure. Male offspring pup body weight was significantly decreased (p<0.05) in males at day 21 in both generations. **The offspring toxicity LOAEL is 39 mg/kg/day based on decreased body weights in both generations of males at PND 21. The offspring toxicity NOAEL is 3.78 mg/kg/day.**

This study is classified **Acceptable - Guideline** and satisfies the guideline requirement for a 2-generation reproductive study (OPPTS 870.3800, §83-4) in the rat.

## **5.5 Additional Information from Literature Sources**

An open literature publication (MRID 45166902, discussed above under "Acute RfD, Study #4") has demonstrated that exposure of a lactating dam to ATR during the days shortly after parturition may result in increased incidence and severity of prostate inflammation in male offspring. Other work from the NHEERL laboratories at EPA has indicated that ATR exposure to immature rats may delay the onset of puberty (Stoker *et al.*, 2000. The Effects of Atrazine on Puberty and Thyroid Function in the Male Wistar Rat: An Evaluation in the Male Pubertal Protocol. Submitted; Laws, *et al.* 2000. The effects of ATR on puberty in female Wistar rats: An evaluation in the protocol for the assessment of pubertal development and thyroid function. Submitted).

The mode of action for these two effects (prostate inflammation and delayed puberty) is believed to be similar to the mode of action described for ATR-associated cancer and involves the CNS neuroendocrine alterations described in the HED CARC document (specifically, neuroendocrine alterations at the hypothalamus).

## **5.6 Determination of Susceptibility**

HIARC concluded that an increased quantitative or qualitative susceptibility was not seen in the developmental or two-generation reproduction studies. However, the studies mentioned above under section 5.5 provide evidence of increased susceptibility.

### **Recommendation for a Developmental Neurotoxicity Study**

#### **5.6.1 Evidence that suggest requiring a Developmental Neurotoxicity study (DNT):**

Special studies and an open literature study mentioned above under section 5.2 indicate a neuroendocrine toxicity in the CNS of rats following ATR exposure.

#### **5.6.2 Evidence that **do not** support a need for a Developmental Neurotoxicity study:**

Evidence of neurotoxicity was seen following ATR exposure. The neurotoxicity seen following ATR exposure is a CNS mode of action supported through a series of registrant submitted studies and studies performed by EPA scientists at NHEERL. A standard DNT is not recommended because ATR's CNS mode of action primarily affects pituitary endocrine function, and the parameters measured in the DNT, i.e., the functional endpoints (motor activity tests, auditory startle tests, and learning and memory tests) may not be sensitive to detect behavioral consequences of this hypothalamic disruption. Certain measures performed in the DNT (such as determination of onset of developmental landmarks and neuropathology) would be useful in examining this CNS neuroendocrine toxicity. However, special studies designed specifically to examine these endpoints would be much more useful in this regard.

Therefore, HIARC determined that a DNT is not required. Instead, the HIARC recommended that studies examining the specific CNS alterations described in the studies conducted by the registrant and the Agency's NHEERL labs be performed.

## **6 HAZARD CHARACTERIZATION**

ATR is herbicide most commonly used on corn and sorghum to control broadleaf grasses. The toxicological database for ATR is complete, and acceptable. ATR has low acute toxicity and is not a dermal sensitizer.

Guideline subchronic, dermal, chronic, developmental, and reproduction studies did not indicate any particular target organ for toxicity except pituitary and mammary gland tumors in females of Sprague Dawley rats). However, special studies have indicated that ATR disrupts hypothalamic-pituitary gonadal axis via a CNS target. Neuroendocrine alterations of the hypothalamic-pituitary axis of rodents following ATR exposure have been described both in studies submitted by the registrant and in studies conducted by EPA labs. These alterations are seen in chronic studies at low doses and in shorter term studies at higher doses. ATR's effect on ovarian cycling and the pre-ovulatory LH surge (as well as its effects on pregnancy, puberty, suckling induced PRL release which leads to prostatitis) are viewed as neuroendocrinopathies or biomarkers indicative of ATR's ability to alter hypothalamic-pituitary function in general. It should be noted that ATR's neuroendocrine effects have been demonstrated in several strains of rats (Sprague Dawley, Long Evans, Wistar).

The mutagenicity database for ATR is extensive and has indicated that ATR is not mutagenic. Special studies have also been conducted to determine the estrogenic potential of ATR and these studies have demonstrated that ATR lacks direct estrogenic activity.

The Cancer Assessment Review Committee (CARC) has evaluated ATR and a December 2000 memorandum has been prepared that considers the final report of the FIFRA Science Advisory Panel June 28 - 30, 2000. The CARC concludes that ATR should be classified as "unlikely to be carcinogenic to humans".

## **7      DATA GAPS**

There are no data gaps for ATR according to the Subdivision F Guideline requirements. HIARC recommends, *but does not require*, that special studies examining ATR's associations with delayed puberty and prostatitis in offspring of dams exposed shortly after parturition, be conducted. Should such studies be conducted, it is recommended that study protocols be approved by HED prior to commencement of any such study.

In addition, HIARC recommends, but does not require, that special studies examining the CNS alterations following ATR exposure be performed.

## 8 ACUTE TOXICITY

### Acute Toxicity of Atrazine

<b>Guideline No.</b>	<b>Study Type</b>	<b>MRIDs #</b>	<b>Results</b>	<b>Toxicity Category</b>
81-1	Acute Oral	Acc 230303	LD <sub>50</sub> = 1,869 mg/kg (M+F combined)	III
81-2	Acute Dermal	Acc 230303	LD <sub>50</sub> > 2,000 mg/kg (M+F combined)	III
81-3	Acute Inhalation	430165-02	LC <sub>50</sub> > 5.8 mg/L (M+F combined)	IV
81-4	Primary Eye Irritation	Acc 230303	PIS= 0.0/110	IV
81-5	Primary Skin Irritation	Acc 230303	PIS= 0.2/8.0	IV
81-6	Dermal Sensitization	001051-31	Non-sensitizing	IV
81-7	Acute Neurotoxicity	none	Not Applicable	—

## 9 SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

The doses and toxicological endpoints selected for various exposure scenarios are summarized below.

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY
Acute Dietary	NOAEL= 10  UF = 100	Delayed ossification of certain cranial bones	Developmental toxicity study in the rat & rabbit (weight of evidence from four studies)
	<b>Acute RfD</b> = 0.1 mg/kg/day		
Chronic Dietary	NOAEL = 1.8 UF = 100	Attenuation the pre-ovulatory LH surge as a biomarker indicative of hypothalamic disruption	Six-month LH surge study in the rat
		<b>Chronic RfD</b> = 0.018 mg/kg/day	
Incidental Oral, Short-Term	NOAEL= 10	Decreased body weight during the first five days of dosing in the dams	Developmental toxicity study in the rat
Incidental Oral, Intermediate-Term	NOAEL= 1.8	Attenuation the pre-ovulatory LH surge as a biomarker indicative of hypothalamic disruption	Six-month LH surge study in the rat
Dermal, Short-Term <sup>a</sup>	NOAEL= 360  (NOAEL from study was 100 mg/kg/day. Multiplied by the rat:human dermal penetration factor of 3.6 = 360 mg/kg/day)	reductions in food consumption, mean body weight, body weight gain, increases in absolute/relative spleen weights and slight changes in excretion ( <i>i.e.</i> few and/or mucoid feces).	21-day dermal toxicity study
Dermal, Intermediate-Term <sup>a</sup>	NOAEL= 1.8	Attenuation the pre-ovulatory LH surge as a biomarker indicative of hypothalamic disruption	Six-month LH surge study in the rat
Dermal, Long-Term <sup>b</sup>	NOAEL= 1.8	Attenuation the pre-ovulatory LH surge as a biomarker indicative of hypothalamic disruption	Six-month LH surge study in the rat
Inhalation, Short-Term <sup>c</sup>	NOAEL= 10	Decreased body weight during the first five days of dosing in the dams	Developmental toxicity study in the rat
Inhalation, Intermediate-Term <sup>c</sup>	NOAEL= 1.8	Attenuation the pre-ovulatory LH surge as a biomarker indicative of hypothalamic disruption	Six-month LH surge study in the rat

Inhalation, Long-Term <sup>c</sup>	NOAEL= 1.8	Same as intermediate term	Same as intermediate term
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a The rat:human dermal penetration factor of 3.6 is applied to this scenario only.

b Dermal absorption rate = 6%

c Convert from oral dose using an inhalation absorption rate = 100% default